

Slow-binding inhibition of acetylcholinesterase by an alkylammonium derivative of 6-methyluracil: Mechanism and possible advantages for myasthenia gravis treatment

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Abstract

© 2016 The Author(s). Published by Portland Press Limited on behalf of the Biochemical Society. Inhibition of human AChE (acetylcholinesterase) and BChE (butyrylcholinesterase) by an alkylammonium derivative of 6-methyluracil, C-547, a potential drug for the treatment of MG (myasthenia gravis) was studied. Kinetic analysis of AChE inhibition showed that C-547 is a slow-binding inhibitor of type B, i.e. after formation of the initial enzyme•inhibitor complex ($K_i = 140$ pM), an induced-fit step allows establishment of the final complex ($K_i = 22$ pM). The estimated k_{off} is low, 0.05 s^{-1} . On the other hand, reversible inhibition of human BChE is a fast-binding process of mixed-type ($K_i = 1.77 \text{ }\mu\text{M}$; $K_i = 3.17 \text{ }\mu\text{M}$). The crystal structure of mouse AChE complexed with C-547 was solved at $3.13 \text{ }\text{\AA}$ resolution. The complex is stabilized by cation- π , stacking and hydrogenbonding interactions. Molecular dynamics simulations of the binding/dissociation processes of C-547 and C-35 (a noncharged analogue) to mouse and human AChEs were performed. Molecular modelling on mouse and human AChE showed that the slow step results from an enzyme conformational change that allows C-547 to cross the bottleneck in the active-site gorge, followed by formation of tight complex, as observed in the crystal structure. In contrast, the related non-charged compound C-35 is not a slow-binding inhibitor. It does not cross the bottleneck because it is not sensitive to the electrostatic driving force to reach the bottom of the gorge. Thus C-547 is one of the most potent and selective reversible inhibitors of AChE with a long residence time, $\tau_i = 20$ min, longer than for other reversible inhibitors used in the treatment of MG. This makes C-547 a promising drug for the treatment of this disease.

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Keywords

6-methyluracil, Acetylcholinesterase, Butyrylcholinesterase, Molecular modelling, Slow-binding inhibition, X-ray structure.